FIG. IA

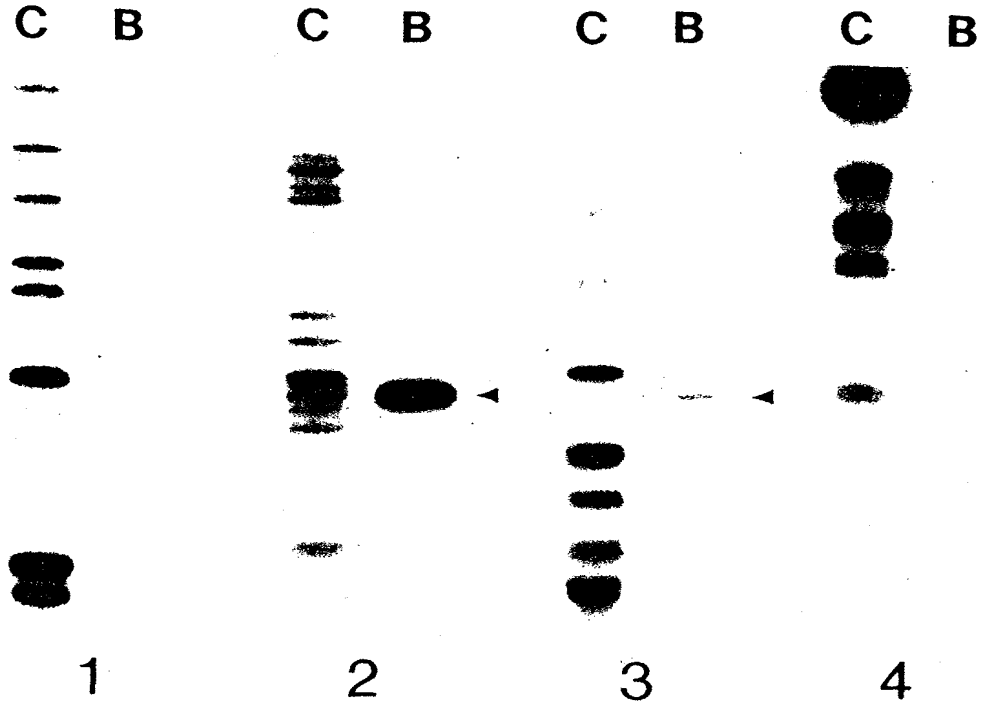


FIG. IB

Host cell:	HeLa			BSC40		
p53:	-	+	+	-	+	+
p53 Ab:	+	+	-	+	+	-

FIG. 2A

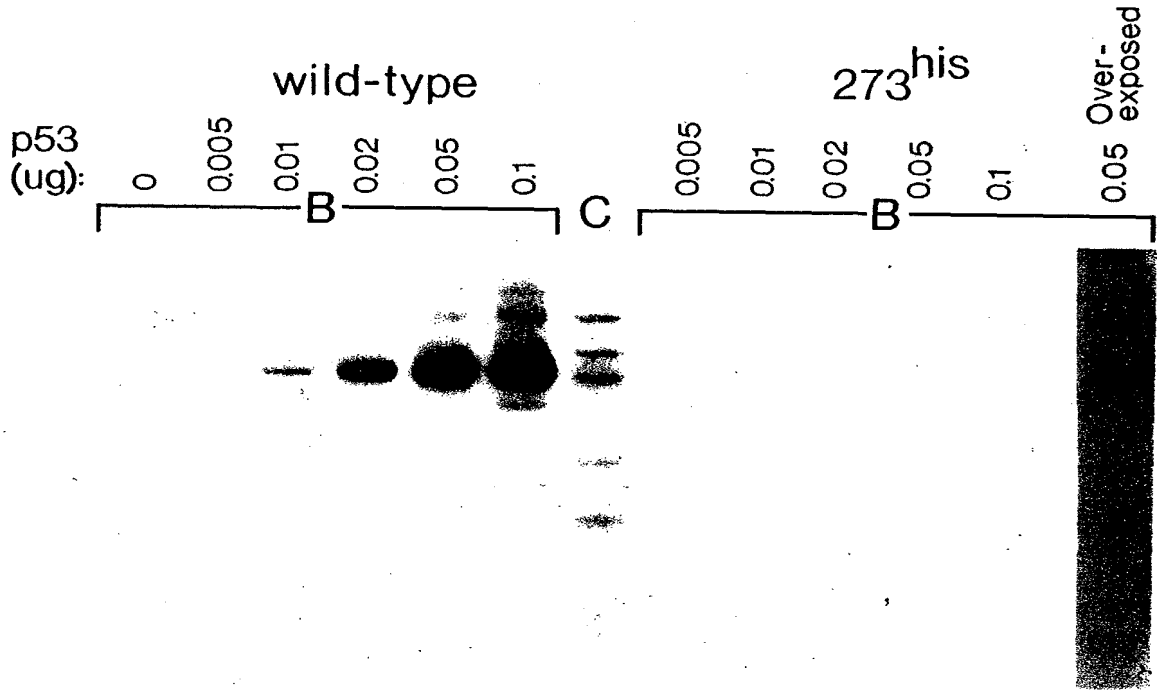

wild-type
273his
Over-exposed
p53 (ug):
0
0.005
0.01
0.02
0.05
0.1
B
C
0.005
0.01
0.02
0.05
0.1
0.05
B

FIG. 2B

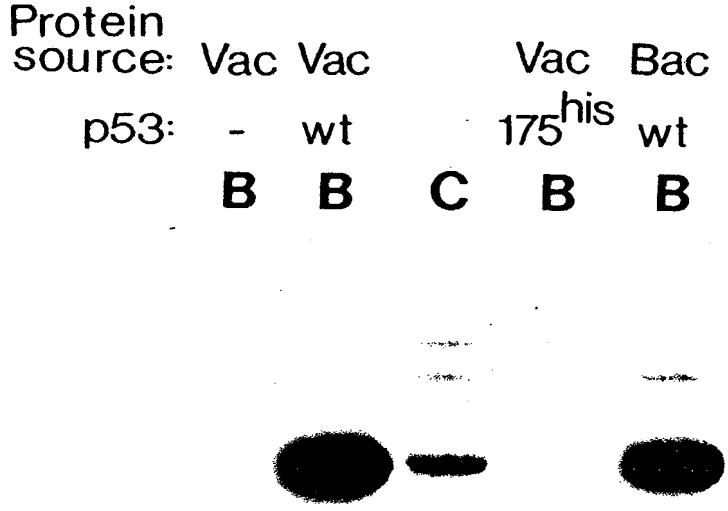

Protein source:
Vac
Vac
Vac
Bac
p53:
-
wt
175his
wt
B
B
C
B
B

FIG. 3A

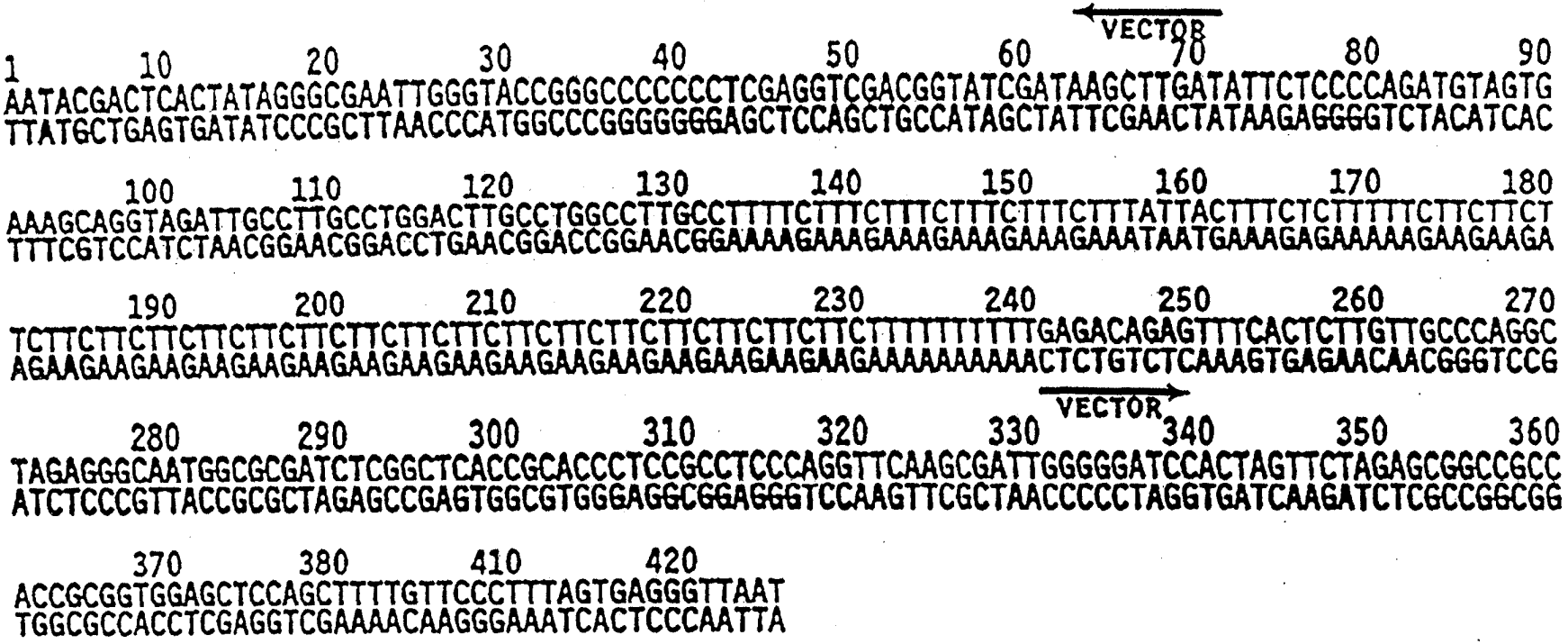

VECTOR
1 10 20 30 40 50 60 70 80 90
AATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCCTCGAGGTCGACGGTATCGATAAGCTTGATATTCTCCCCAGATGTAGTG
TTATGCTGAGTGATATCCCGCTTAACCCATGGCCCGGGGGGGGAGCTCCAGCTGCCATAGCTATTCGAACTATAAGAGGGGTCTACATCAC
100 110 120 130 140 150 160 170 180
AAAGCAGGTAGATTGCCTTGCCTGGACTTGCCTGGCCTTGCCTTTTCTTTCTTTCTTTCTTTCTTTATTACTTTCTCTTTTTCTTCTTCT
TTTCGTCCATCTAACGGAACGGACCTGAACGGACCGGAACGGAAAAGAAAGAAAGAAAGAAAGAAATAATGAAAGAGAAAAAGAAGAAGA
190 200 210 220 230 240 250 260 270
TCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTCTTTTTTTTTTTGAGACAGAGTTTCACTCTTGTTGCCCAGGC
AGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAAAAAAAAAACTCTGTCTCAAAGTGAGAACAACGGGTCCG
VECTOR
280 290 300 310 320 330 340 350 360
TAGAGGGCAATGGCGCGATCTCGGCTCACCGCACCCTCCGCCTCCCAGGTTCAAGCGATTGGGGGATCCACTAGTTCTAGAGCGGCCGCC
ATCTCCCGTTACCGCGCTAGAGCCGAGTGGCGTGGGAGGCGGAGGGTCCAAGTTCGCTAACCCCCTAGGTGATCAAGATCTCGCCGGCGG
370 380 410 420
ACCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAAT
TGGCGCCACCTCGAGGTCGAAAACAAGGGAAATCACTCCCAATTA

FIG. 3B

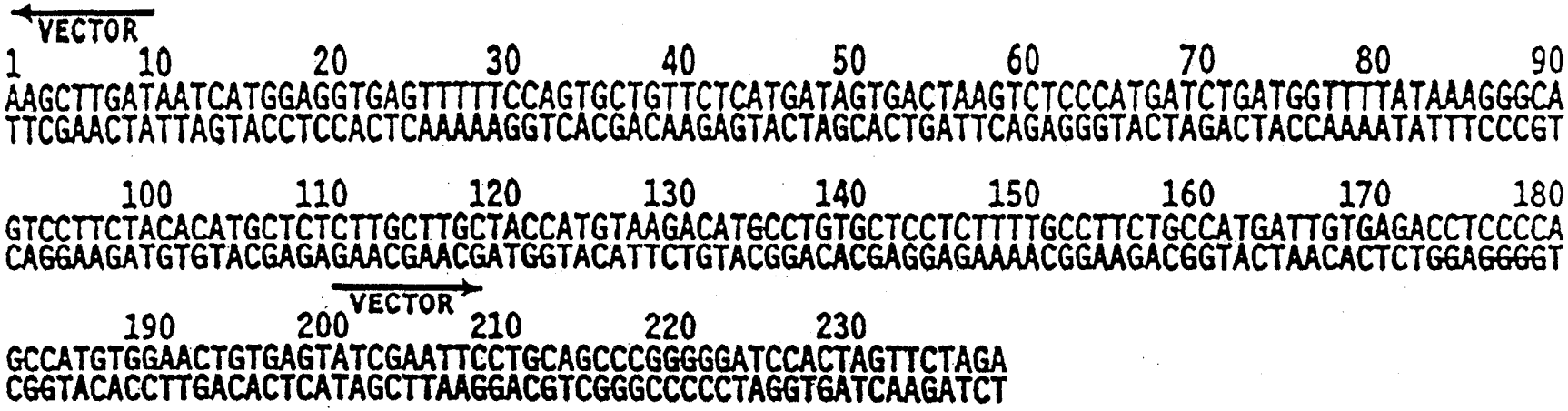

VECTOR
1 10 20 30 40 50 60 70 80 90
AAGCTTGATAATCATGGAGGTGAGTTTTTCCAGTGCTGTTCTCATGATAGTGACTAAGTCTCCCATGATCTGATGGTTTTATAAAGGGCA
TTCGAACTATTAGTACCTCCACTCAAAAAGGTCACGACAAGAGTACTAGCACTGATTCAGAGGGTACTAGACTACCAAAATATTTCCCGT
100 110 120 130 140 150 160 170 180
GTCCTTCTACACATGCTCTCTTGCTTGCTACCATGTAAGACATGCCTGTGCTCCTCTTTTGCCTTCTGCCATGATTGTGAGACCTCCCCA
CAGGAAGATGTGTACGAGAGAACGAACGATGGTACATTCTGTACGGACACGAGGAGAAAACGGAAGACGGTACTAACACTCTGGAGGGGT
VECTOR
190 200 210 220 230
GCCATGTGGAACTGTGAGTATCGAATTCCTGCAGCCCGGGGGATCCACTAGTTCTAGA
CGGTACACCTTGACACTCATAGCTTAAGGACGTCGGGCCCCCTAGGTGATCAAGATCT

FIG. 4A

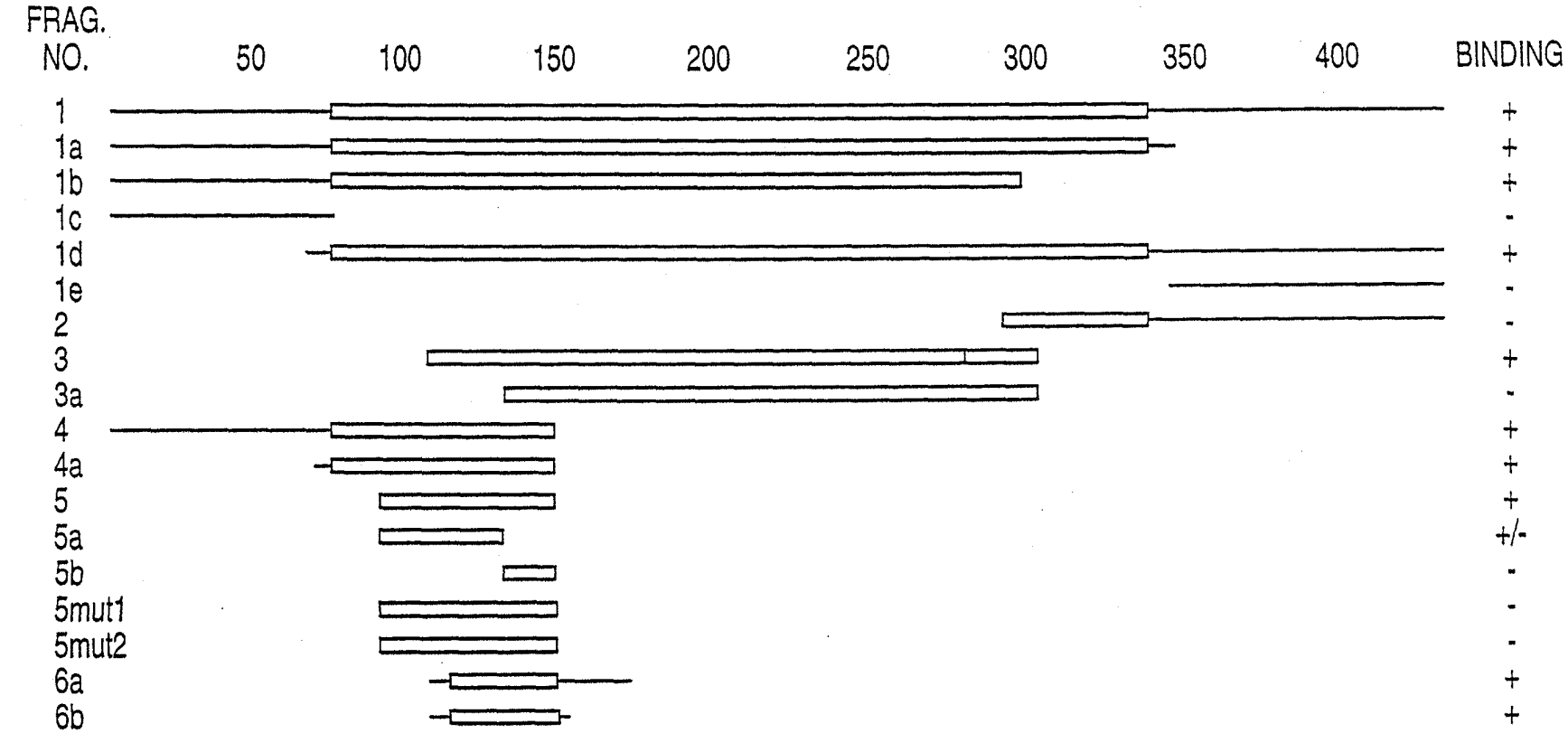

FRAG. NO.
50
100
150
200
250
300
350
400
BINDING
1 +
1a +
1b +
1c -
1d +
1e -
2 -
3 +
3a -
4 +
4a +
5 +
5a +/-
5b -
5mut1 -
5mut2 -
6a +
6b +

FIG. 4B

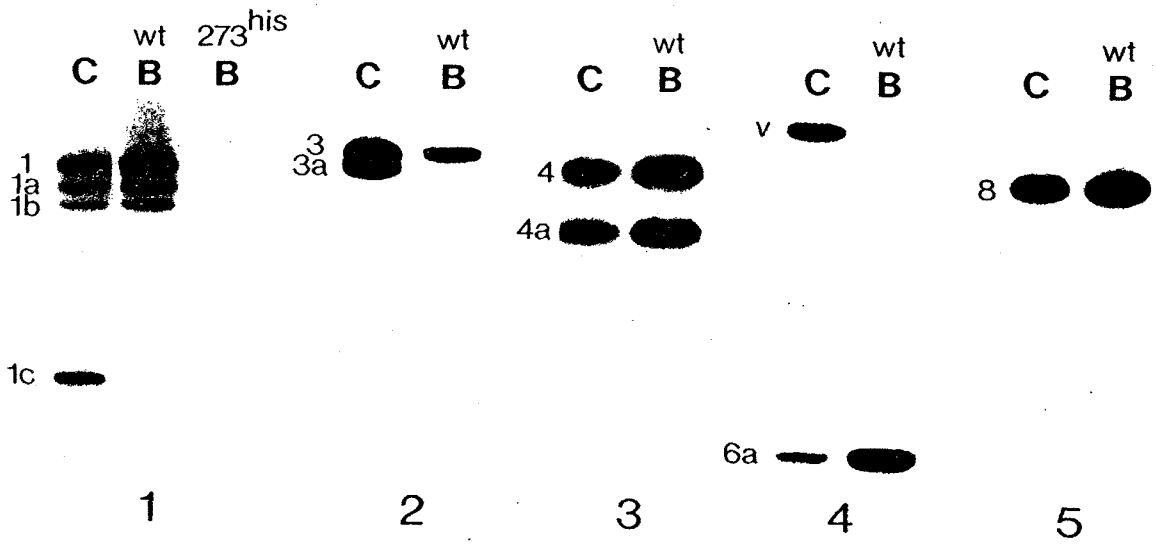


FIG. 5A

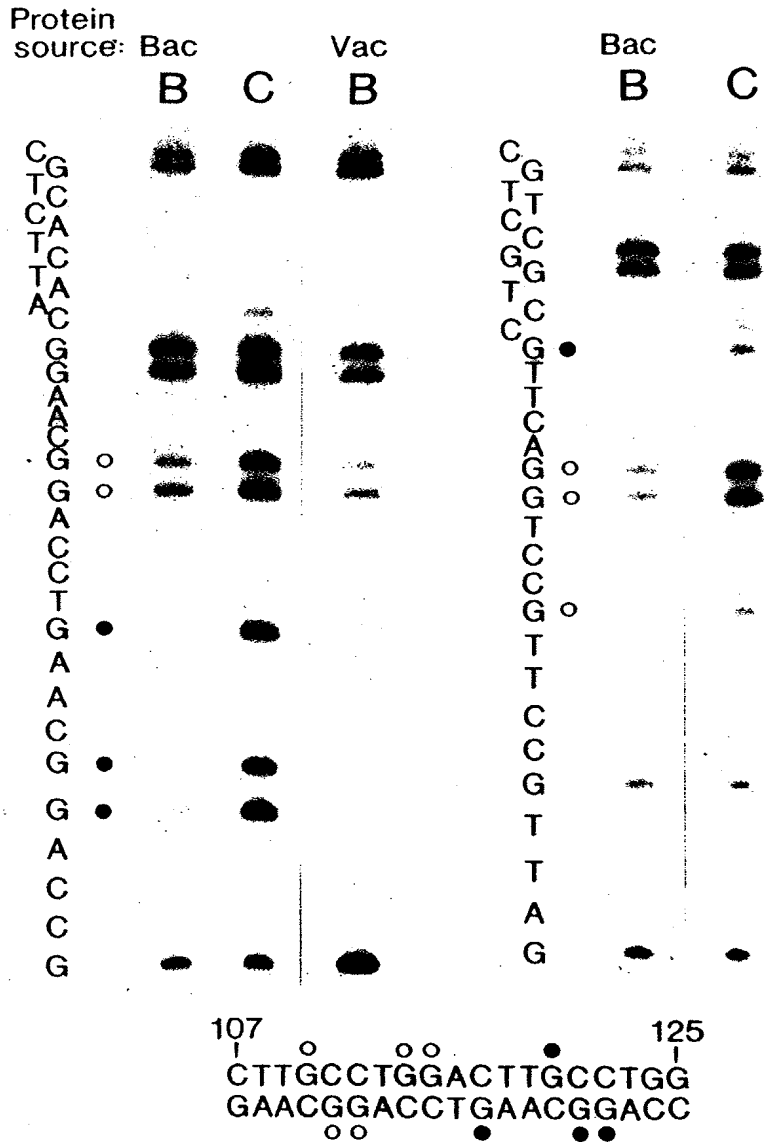
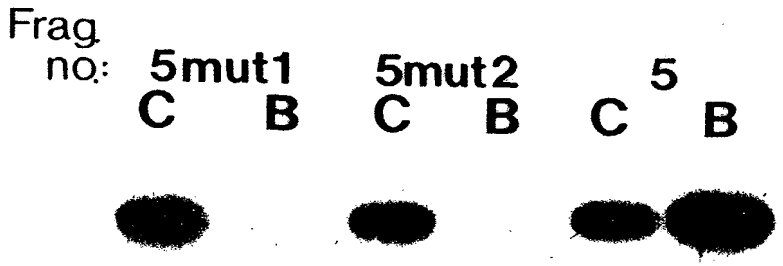

Protein source:
Bac
Vac
Bac
B
C
B
B
C
107
125
CTTGCCTGGACTTGCCTGG
GAACGGACCTGAACGGACC

FIG. 5B



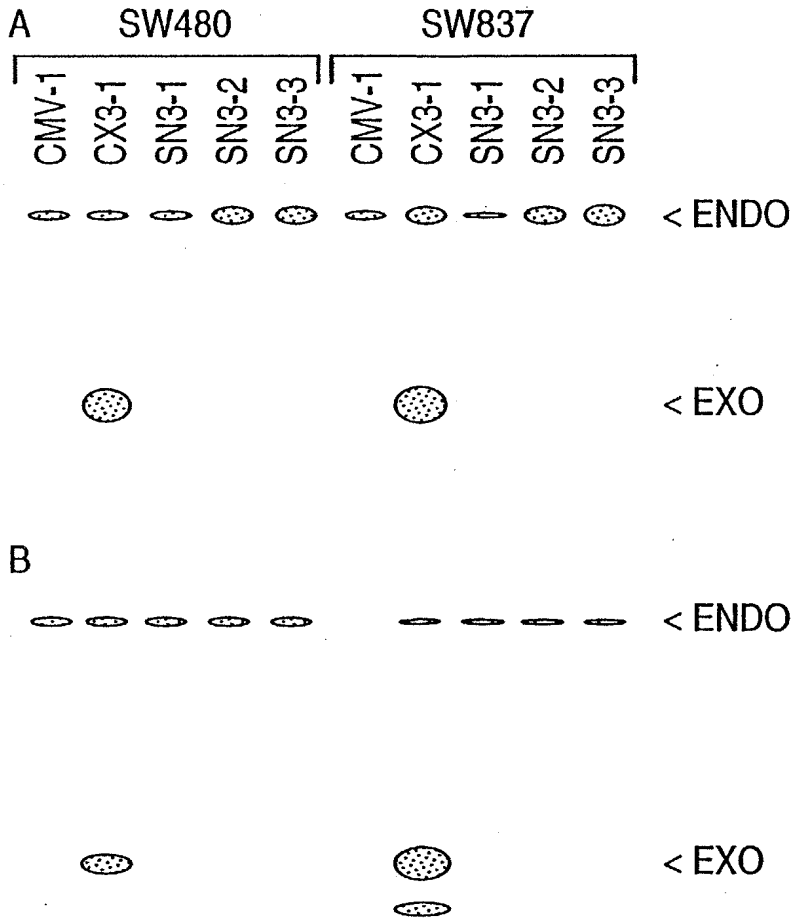

A
SW480
SW837
CMV-1
CX3-1
SN3-1
SN3-2
SN3-3
CMV-1
CX3-1
SN3-1
SN3-2
SN3-3
< ENDO
< EXO
B
< ENDO
< EXO

FIG. 6

FIG. 7A

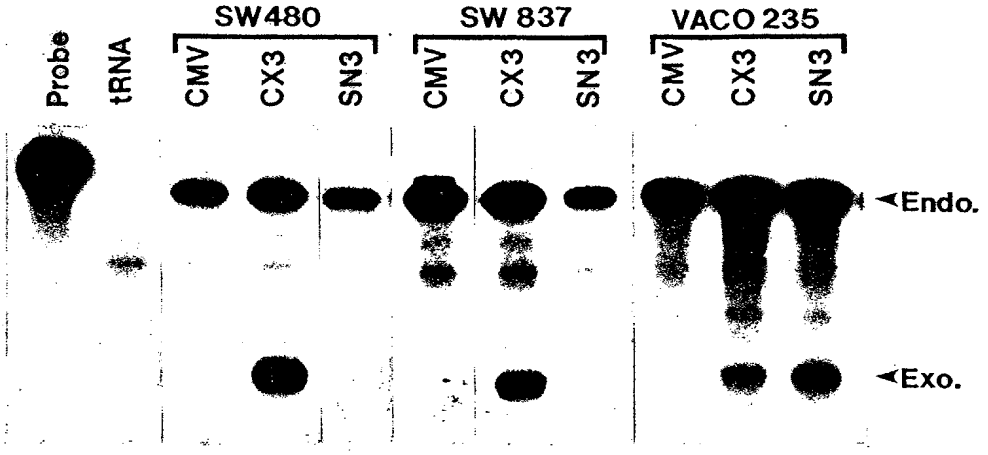

Probe
tRNA
SW480
SW 837
VACO 235
CMV
CX3
SN3
CMV
CX3
SN3
CMV
CX3
SN3
Endo.
Exo.

FIG. 7B

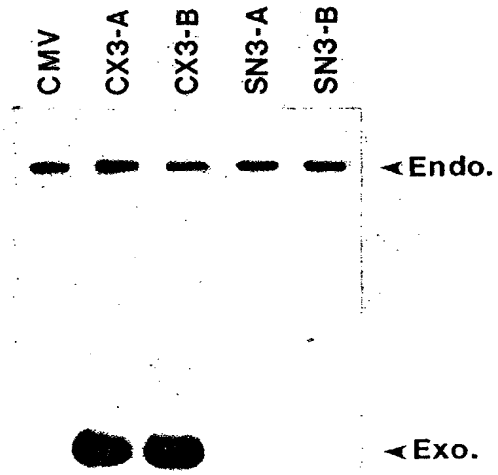

CMV
CX3-A
CX3-B
SN3-A
SN3-B
Endo.
Exo.

FIG. 8A

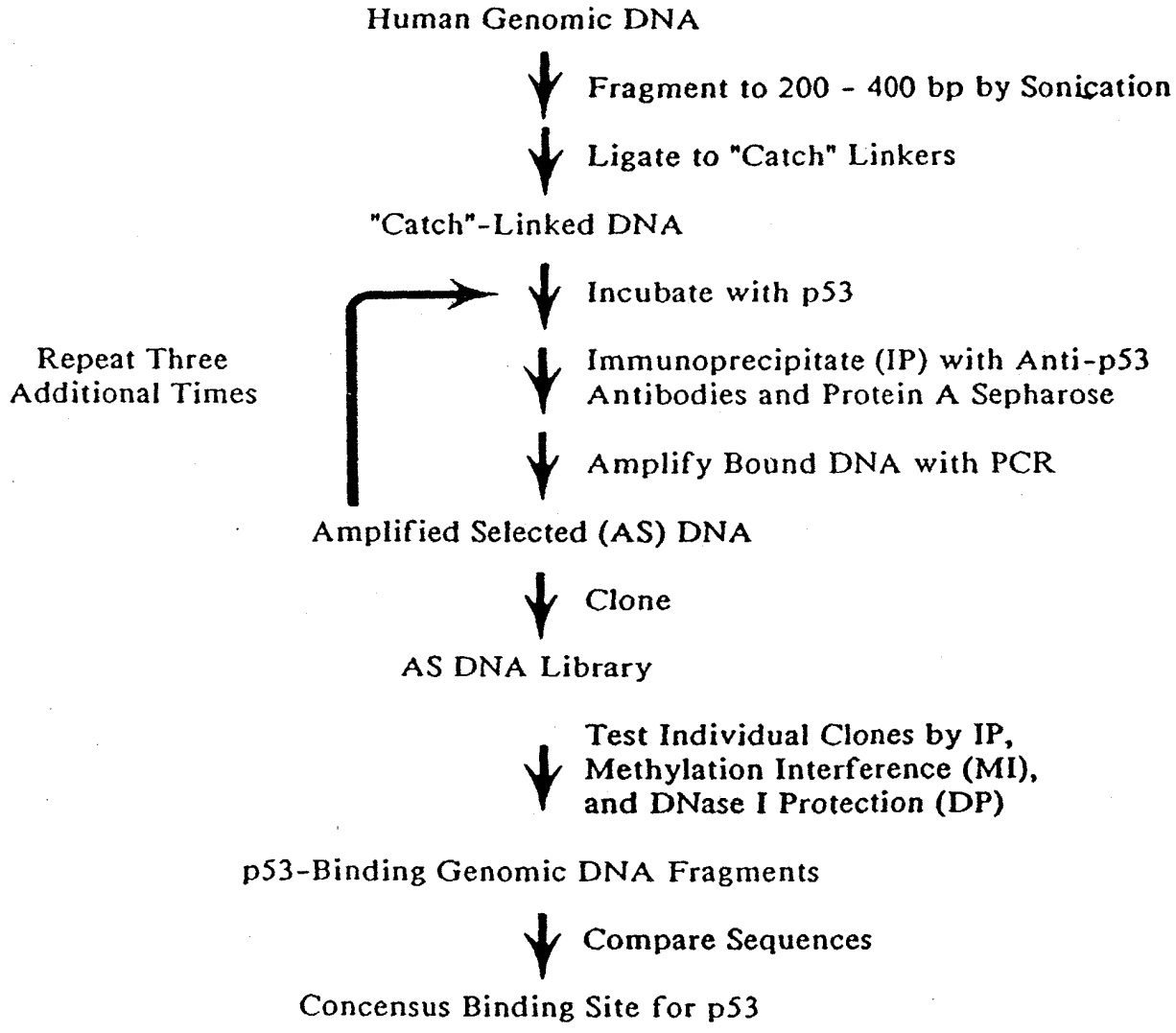

Human Genomic DNA
Fragment to 200 - 400 bp by Sonication
Ligate to "Catch" Linkers
"Catch"-Linked DNA
Incubate with p53
Immunoprecipitate (IP) with Anti-p53 Antibodies and Protein A Sepharose
Amplify Bound DNA with PCR
Repeat Three Additional Times
Amplified Selected (AS) DNA
Clone
AS DNA Library
Test Individual Clones by IP, Methylation Interference (MI), and DNase I Protection (DP)
p53-Binding Genomic DNA Fragments
Compare Sequences
Concensus Binding Site for p53

FIG. 8B

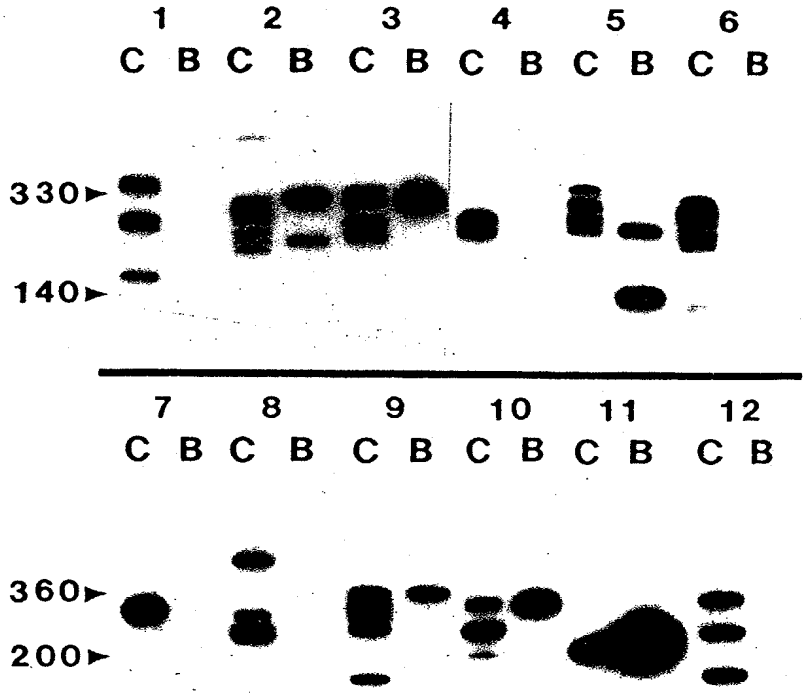

1 2 3 4 5 6
C B C B C B C B C B C B
330►
140►
7 8 9 10 11 12
C B C B C B C B C B C B
360►
200►

FIG. 9

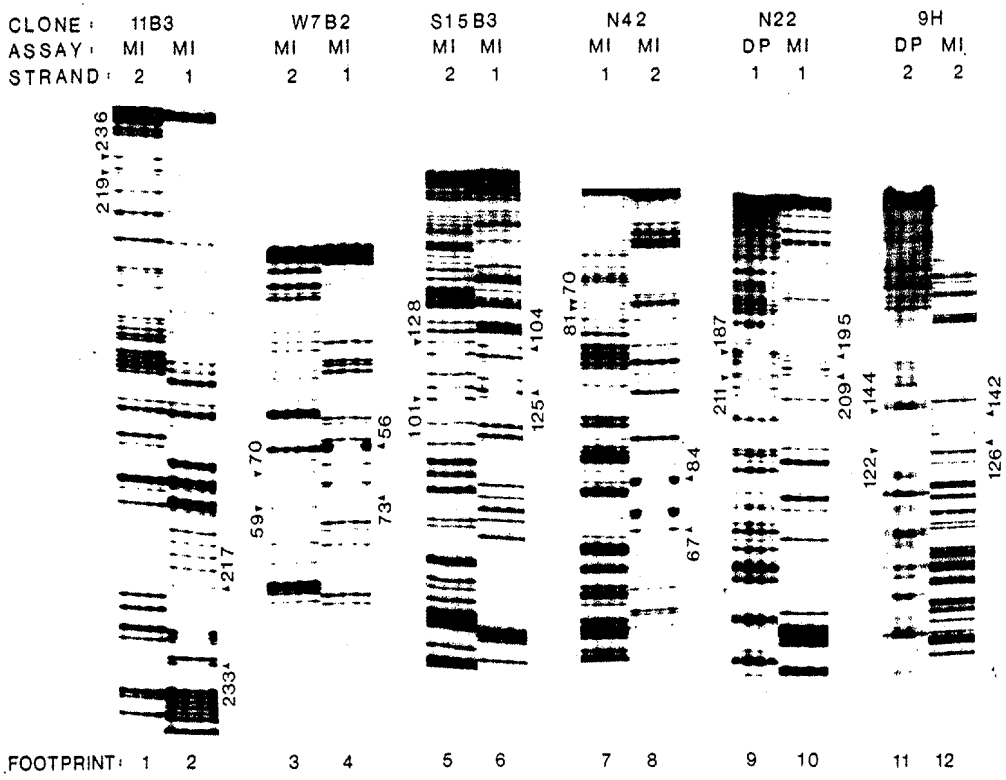

CLONE: 11B3 W7B2 S15B3 N42 N22 9H
ASSAY: MI MI MI MI MI MI MI MI DP MI DP MI
STRAND: 2 1 2 1 2 1 1 2 1 1 2 2
219 236 217 233
59 70 56 73
101 128 104 125
81 70 84 67
211 187 209 195
122 144 126 142
FOOTPRINT: 1 2 3 4 5 6 7 8 9 10 11 12

FIG. 10A

	Clone	Size (bp)	5'-bp	nnnnnnnnnnnnnnn	R R R C W W G Y Y Y	nnnnnnnnnnnnn
1.	S57	295	144	cgacctgtcacaccg	G G G C c T G T C a	
2.	N22	357	178	attttcaccatgctt	c t G C A T G T C T	
3.	11A2	387	317	ccccatcctccactg	A A A C AaT G C C C	
4.	W211	249	119	tttgtcctaccatcc	A G G C A T G C C T	
5.	W7B2	139	41	tatctgtgcagctgt	G G G C A T G T T T	t
6.	3H	126	50	aactagatccttttc	A G A C A T G T T a	
7.	8A	483	445	gctggtgcacaagag	t G A C A T G T C C	
8.	532	335	229	catcatgccacctgc	A G G C A T G T T C	tggat
9.	64A2	349	120	caaaccagggtgtct	t G A C T T G C C T	atcctgggaggt
10.	W7A1	264	124	gccaaacataaccac	c A G C T G C C a	
11.	S61	202	1	c	c A A C T T G T C T	attctgtgttgat
12.	11B3	248	201	actgttgatgatgaa	A G A C A A G C C T	a
13.	N42	248	49	gcagtgtggtggagg	A A A C A A G C C C	a
14.	S201	326	164	tgttcatacctgtcc	A c A C T T G T C T	
15.	S15B3	248	83	ctttaattcagttgt	A A A C A T GaC T T	gttcattata
16.	S592I	254	39	ctcagttctcagctg	G G A C T T G C C C	
17.	S592II	254	130	tgcctcagcaccttc	A G G t TcT G C C	
18.	2Nb	470	42	gcctttgttgtgccc	t G A C T T G C C C	
19.	9H	467	108	gtattctcttttcct	A A G C A T G C C T	
20.	CBE10d	425	89	tgaaagcaggtagat	t G c C T T G C C T	

Combined Nucleotide Usage (%) within the Two Monomers of the Consensus Binding Site:

FIG. 10

FIG. 10A	FIG. 10B
FIG. 10C	FIG. 10D

FIG. 10B

R R R C W W G Y Y Y	nnnnnnnnnnnnnnn	3'-bp
c A G C A T GaC C T	acctgtcacaccggg	194
A G G C A A G T C a	ccttctccactggcc	227
A G A C T T G T C T	ctccggcctgaatga	367
T T G C C T	cactcgttatttcct	164
A G G C A A G C T T	cctgtgctagttccc	91
t A A C A A G T C a	gtacaagtttatttt	99
c G A C g T G T T T	tgtc	483
G G G C T G T C T	tgtgctttgttgttt	282
t G A C A T G T T C	ctccccttcccccctc	181
A G G C A T G C a g	taccacgctcagccc	173
G G A C A T G T T C	ccgtttttggctatt	49
G G G C A g G T C C	tgggggtgggg	248
G G A t g T G C C C	agggcaggctgggac	99
A t A C c T G C C T	acacctgtcttgttt	214
t G A C A T G T T C	aattacaattcgatt	143
t G G C c A G C C C	tggggtcactgctgc	88
G G G C T T G T T C	ctttcctttcagcat	179
A G A C A T G T T T	gggaatgtcttgtgc	91
t G A C T T G T T C	tttcatctcctctga	157
G G A C T T G C C T	ggccttgccttttct	138

FIG. IOC

	5'- R	R	R	C	W	W
A	40	20	55	0	53	15
C	13	3	3	93	8	0
G	23	70	40	0	8	3
T	23	5	0	5	30	82

Synthetic Oligonucleotides:

No.	p53 Binding		
1.	-		A G G a A T t C C T
2.	-		A G G a A T t C C T
3.	-		A G G C A T G T C T
4.	+		A G G C A T G C C T
5.	-		A G G C A A G g C a
6.	+	tgcaggaattcgat	A G G C A T G T C T
7.	+	tgcaggaattcgat	A G G C A T G T C T
8.	+	tgcaggaattcgat	A G A C A T G C C T
9.	-	tgcaggaattcgat	A G G C A T G T C T
10.	-	tgcaggaattcgat	

FIG. 10D

G	Y	Y	Y -3'	
0	0	0	12	A
0	50	68	35	C
100	0	0	3	G
0	50	30	48	T

A G G a A T t C C T

A G G C A T G C C T
A G G C A A G g C a
A G G C A T G T C T atcaagcttatcgat
A G A C A T G C C T atcaagcttatcgat
A G G C A T G T C T atcaagcttatcgat
atcaagcttatcgat
atcaagcttatcgat

FIG. IIA

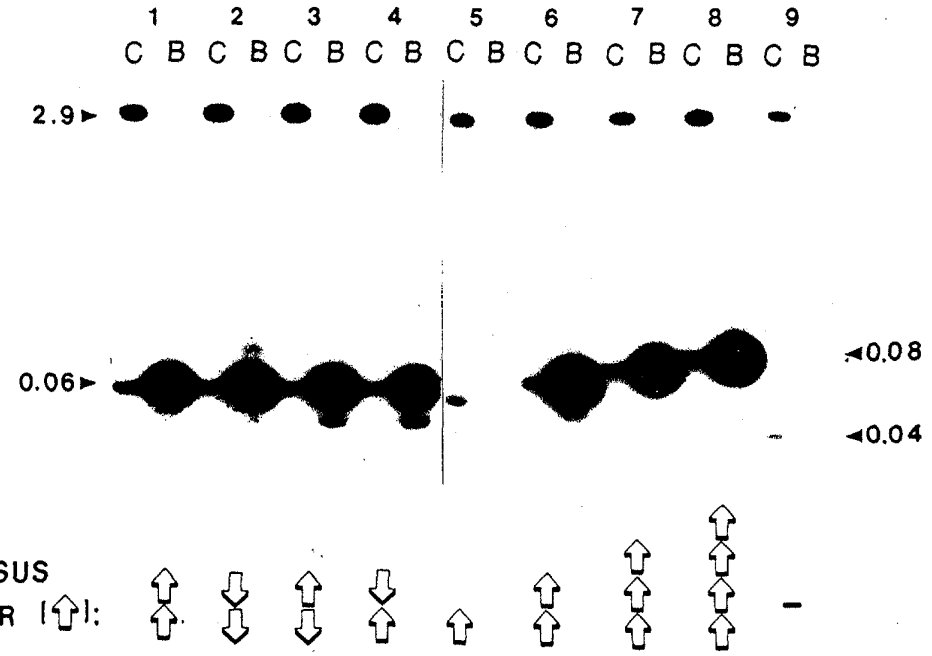

1 2 3 4 5 6 7 8 9
C B C B C B C B C B C B C B C B C B
2.9►
0.06►
◄0.08
◄0.04
CONCENSUS
MONOMER [⇧]:

FIG. IIB

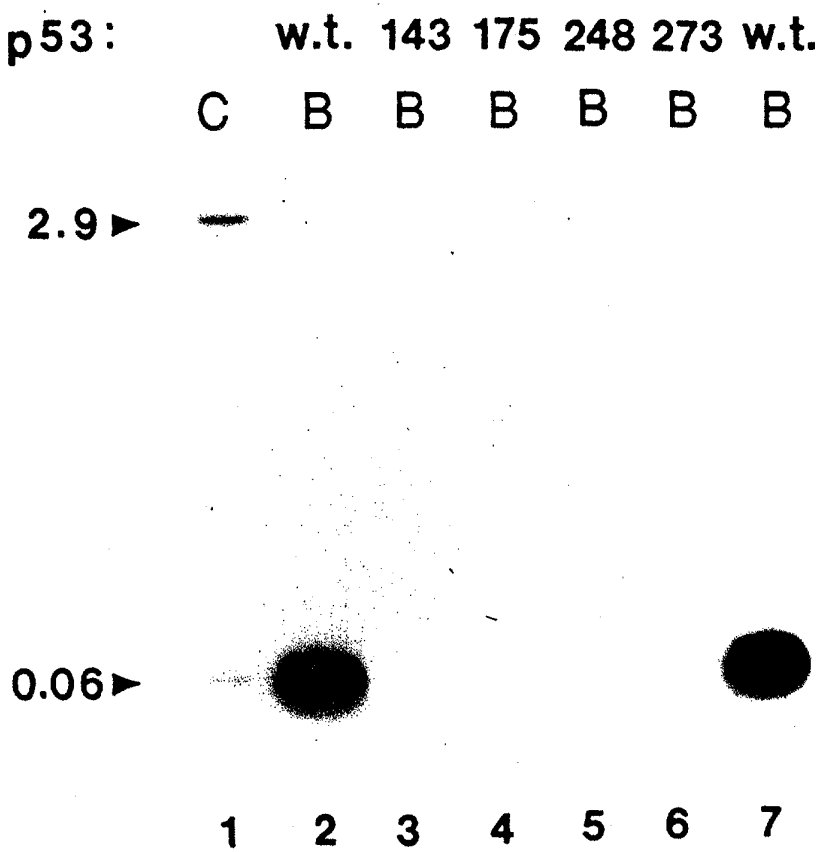


FIG. 12A

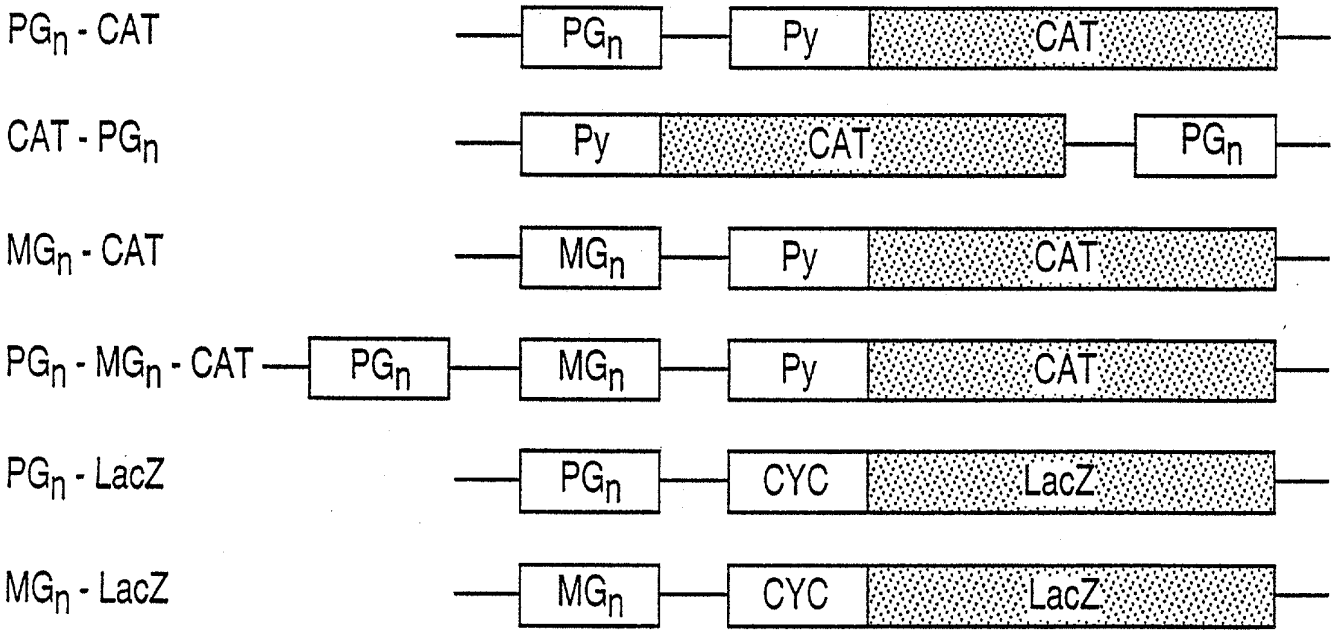

PGn - CAT
PGn
Py
CAT
CAT - PGn
Py
CAT
PGn
MGn - CAT
MGn
Py
CAT
PGn - MGn - CAT
PGn
MGn
Py
CAT
PGn - LacZ
PGn
CYC
LacZ
MGn - LacZ
MGn
CYC
LacZ

FIG. 12B

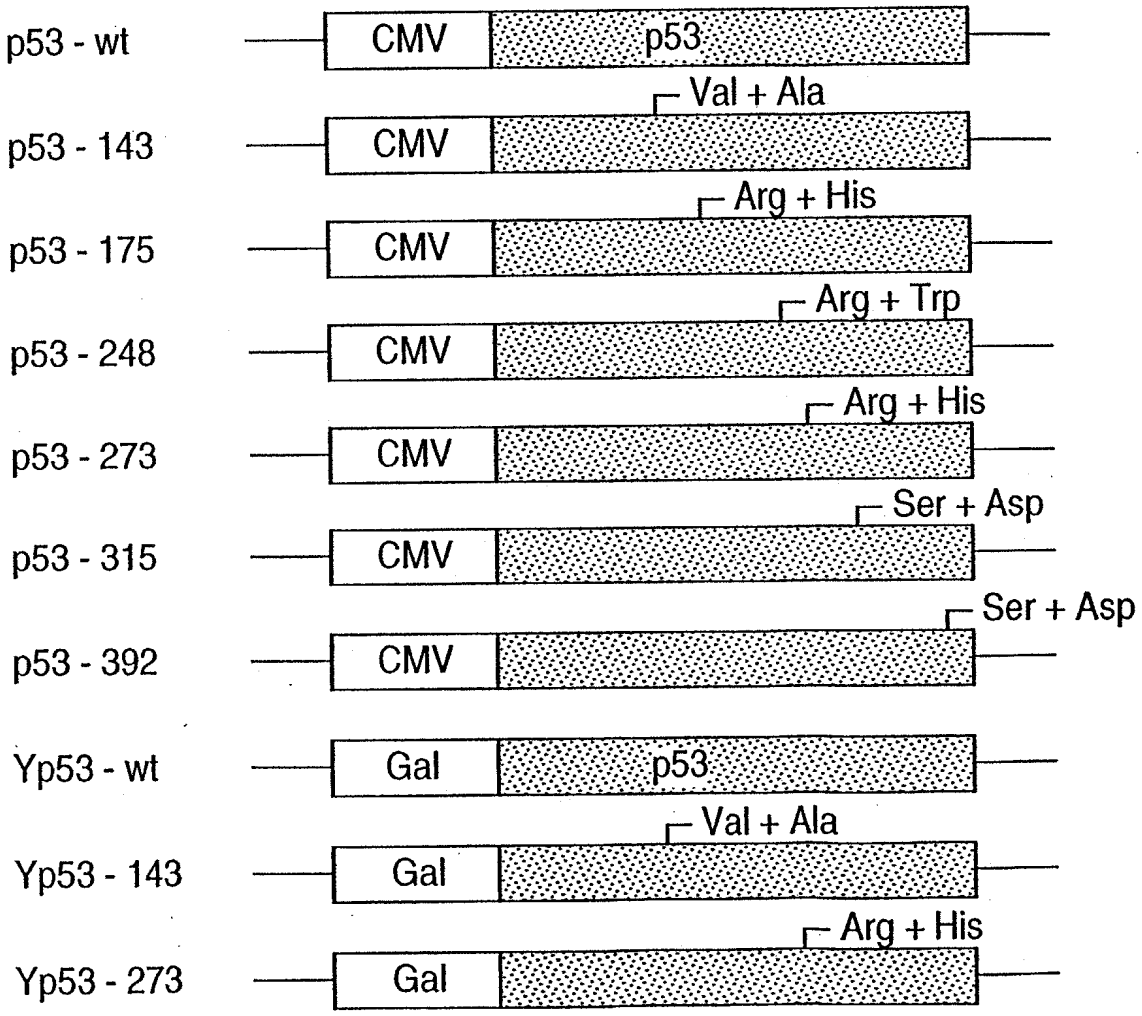

p53 - wt
CMV
p53
Val + Ala
p53 - 143
CMV
Arg + His
p53 - 175
CMV
Arg + Trp
p53 - 248
CMV
Arg + His
p53 - 273
CMV
Ser + Asp
p53 - 315
CMV
Ser + Asp
p53 - 392
CMV
Yp53 - wt
Gal
p53
Val + Ala
Yp53 - 143
Gal
Arg + His
Yp53 - 273
Gal

FIG. 13A

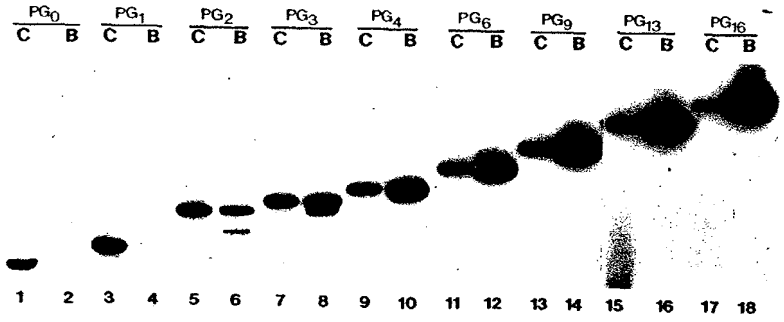


FIG. 13B

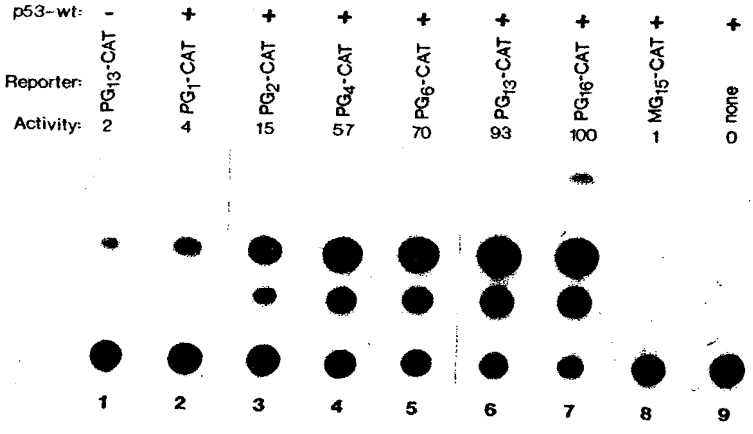


FIG. 14

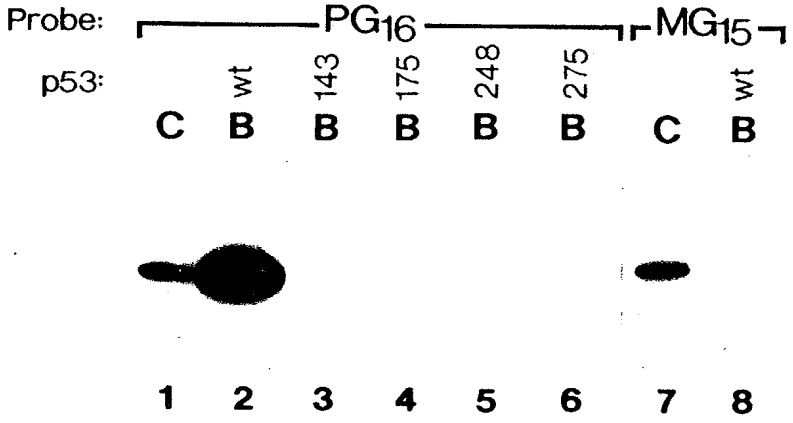

Probe:
PG16
MG15
p53:
C
wt
143
175
248
275
C
wt
B
B
B
B
B
B
B
B
1
2
3
4
5
6
7
8

FIG. 15A

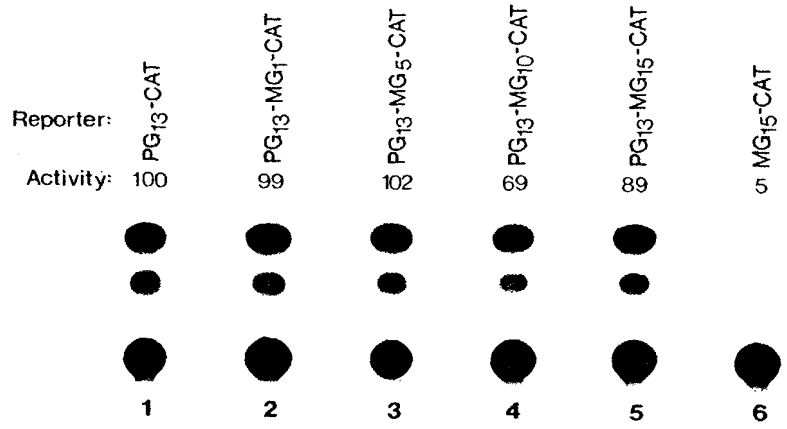


FIG. 15B

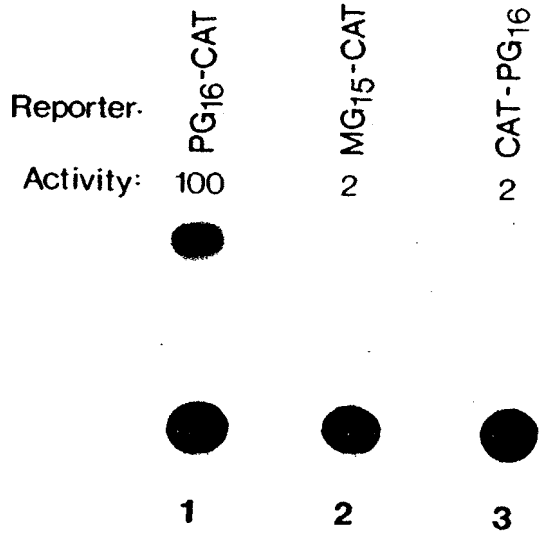


FIG. 16A

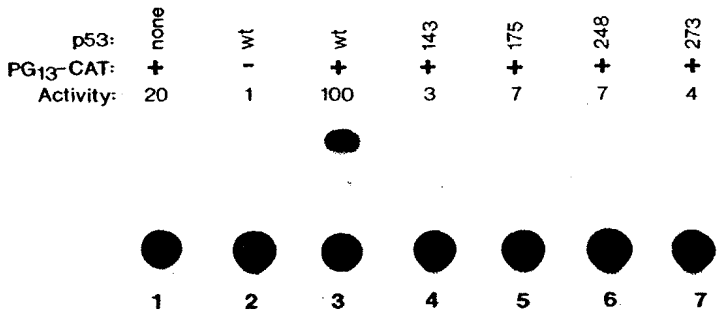


FIG. 16B

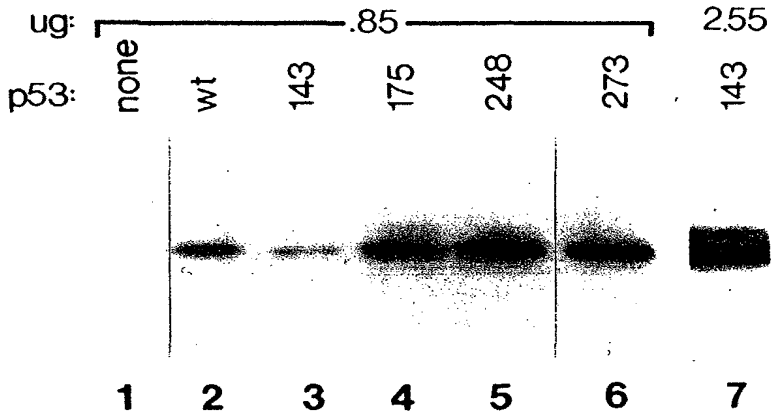


FIG. 17

p53-wt (ug):	0	85	.85	.85	.85
p53-175 (ug):	0	0	0	.85	2.55
PG_{13}-CAT:	+	-	+	+	+
Activity:	12	0	100	44	11

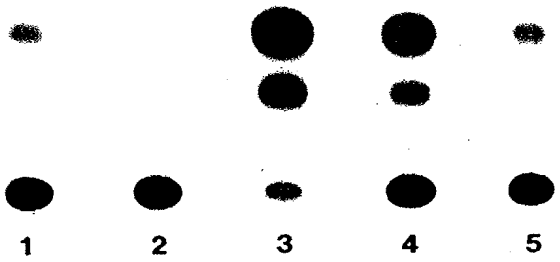


FIG. 18

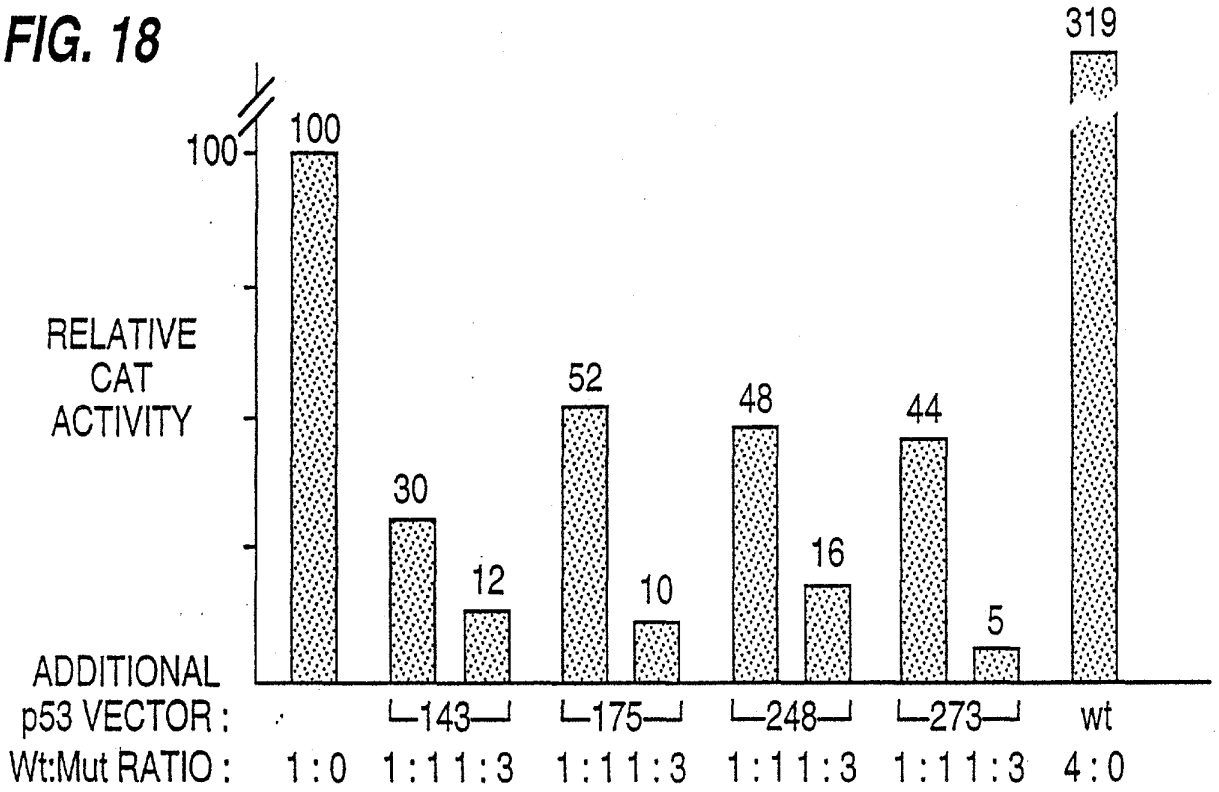
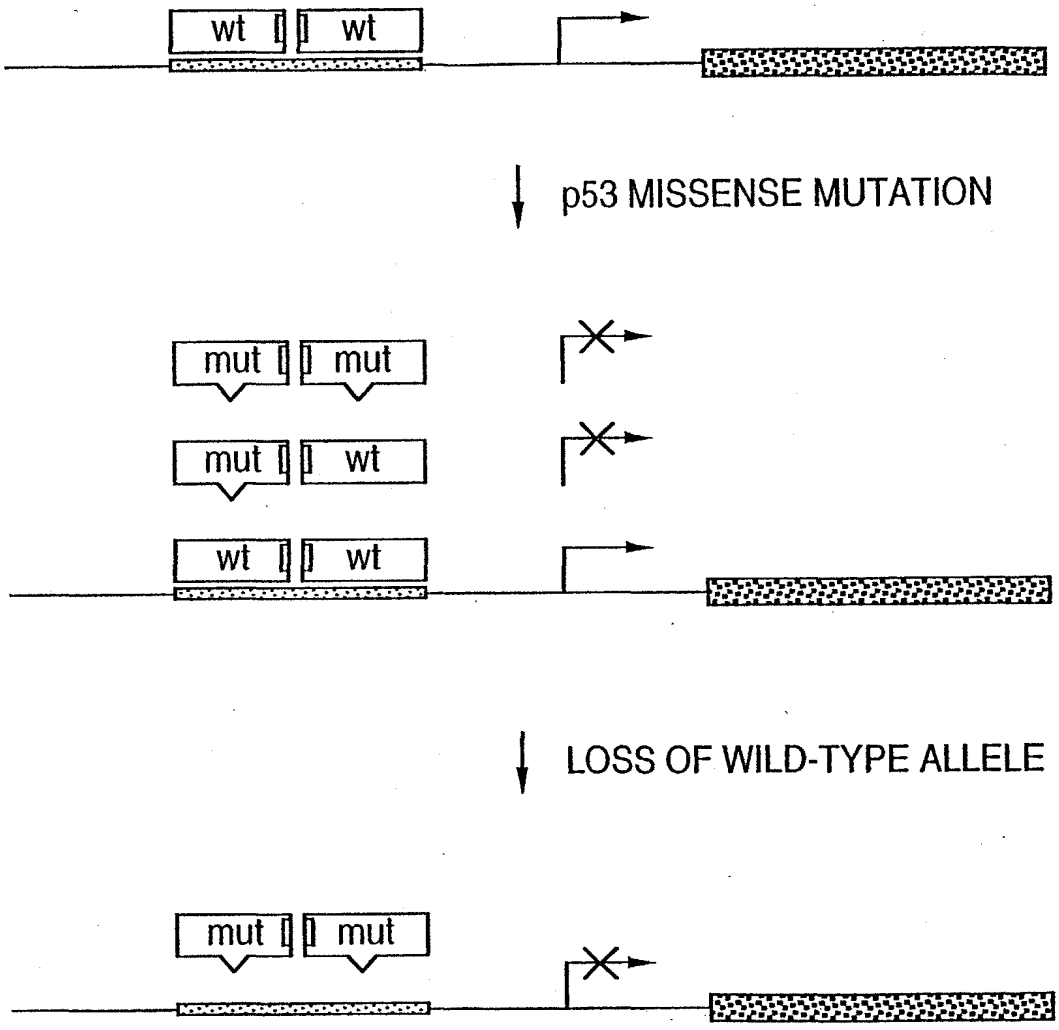

319
100
100
RELATIVE
CAT
ACTIVITY
52
48
44
30
16
12
10
5
ADDITIONAL
p53 VECTOR :
143
175
248
273
wt
Wt:Mut RATIO :
1 : 0
1 : 1
1 : 3
1 : 1
1 : 3
1 : 1
1 : 3
1 : 1
1 : 3
4 : 0

FIG. 19


wt
wt
p53 MISSENSE MUTATION
mut
mut
mut
wt
wt
wt
LOSS OF WILD-TYPE ALLELE
mut
mut